

Two Different Transcription Factors Discriminate the –315C>T Polymorphism of the *FcεRIα* Gene: Binding of Sp1 to –315C and of a High Mobility Group-Related Molecule to –315T¹

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The α -chain is a specific component of Fc ϵ RI, which is essential for the cell surface expression of Fc ϵ RI and the binding of IgE. Recently, two single nucleotide polymorphisms (SNPs) in the α -chain promoter, –315C>T and –66T>C, have been shown by statistic studies to associate with allergic diseases. The effect of –66 SNP on GATA-1-mediated promoter activity has been already indicated. In the present study, to investigate roles of the –315 SNP on the α -chain promoter functions, the transcription activity was evaluated by reporter assay. The α -chain promoter carrying –315T (minor allele) possessed significantly higher transcriptional activity than that of –315C (major allele). EMSA indicated that the transcription factor Sp1, but not Myc-associated zinc finger protein (MAZ), was bound to the –315C allele probe and that a transcription factor belonging to a high mobility group-family bound to the –315T allele probe. The chromatin immunoprecipitation assay suggested that high mobility group 1, 2, and Sp1 bound around –315 of *FcεRIα* genomic DNA in vivo in the human basophil cell line KU812 with –315C/T and in human peripheral blood basophils with –315C/C, respectively. When cell surface expression level of Fc ϵ RI on basophils was analyzed by flow cytometry, basophils from individuals carrying –315T allele expressed significantly higher amount of Fc ϵ RI compared with those of –315C/C. The findings demonstrate that a –315 SNP significantly affects human Fc ϵ RI α -chain promoter activity and expression level of Fc ϵ RI on basophils by binding different transcription factors to the SNP site. *The Journal of Immunology*, 2008, 180: 8204–8210.

The Fc ϵ RI, high-affinity receptor for IgE, mainly expressed on mast cells and basophils plays an important role in mediating allergic reactions caused by allergen-specific IgE Abs. Cross-linking of IgE Abs bound to Fc ϵ RI by allergens induces activation of effector cells, resulting in both the release of chemical mediators and the production of cytokines. Fc ϵ RI is composed of three subunits: the α -chain as IgE-binding subunit, and the β - and γ -chains as the signal transducers. The essential role of the α -chain in Fc ϵ RI-mediated allergic reactions was definitively proved by the absence of an allergic reaction in α -chain-deficient mice (1).

Recently, two single nucleotide polymorphisms (SNPs)⁴ in the human α -chain promoter, –315C>T and –66T>C, have been shown to be associated with allergic diseases (we number nucleotides based on the transcription start site being expressed as + 1 according to the genetic rule; although, in some studies, these nucleotides have been numbered –344 or –335 and –95 using the translation start site as + 1) (2–5). We previously analyzed the effect of a –66T>C SNP on the function of the α -chain gene and found that the occurrence of an additional GATA-motif in the –66T allele resulted in higher affinity to GATA-1, which subsequently caused higher transactivation of the promoter (2). In the present study, the effect of a –315C>T SNP on the gene expression of the α -chain was analyzed. A reporter assay showed that α -chain promoter carrying –315T, which is a minor allele and is found with relatively high frequency in some populations of allergic patients, possessed higher transcriptional activity than that of –315C. EMSA and chromatin immunoprecipitation (ChIP) assay indicated that this SNP is critical for distinct binding of transcription factors. In brief, the –315C allele probe was recognized by Sp1, whereas the –315T allele probe was bound with high mobility group (HMG)-family transcription factors. Peripheral blood basophils from individuals carrying –315T allele exhibited significantly higher amount of Fc ϵ RI on surface compared with those of –315C/C. Although a study regarding this Fc ϵ RI –315 SNP

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⁴ Abbreviations used in this paper: SNP, single nucleotide polymorphism; HMG, high mobility group; ChIP, chromatin immunoprecipitation; MFI, mean fluorescence intensity; LD, linkage disequilibrium.

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was recently published during the preparation of this manuscript (5), we found several discrepancies between that report and the present results. Possible explanations for these differences are also discussed.

Materials and Methods

Cell culture

RBL-2H3, PT18, and KU812 were cultured as described previously (6).

Plasmid construction

Reporter plasmids carrying the luciferase gene under control of the human FcεRI α-chain promoter (−605/+29) with −66T (pGV-B2-αNN0.6−66T) and −66C (pGV-B2-αNN0.6−66C) were constructed as in our previous report (2). Because these two plasmids were identified to have T at −315 by sequencing analysis, site-directed mutagenesis was conducted on −315T allele of the promoter using a QuikChange site-directed mutagenesis kit (Stratagene) to obtain the −315C allele of the promoter. Four reporter plasmids, pGV-B2-αNN0.6−315C/−66T, −315C/−66C, −315T/−66T, and −315T/−66C were prepared.

Luciferase assay

The reporter plasmids were introduced into cells by electroporation as described previously (2). The measurement of luciferase activity and calculation of relative luciferase activity was performed as described previously (7).

EMSA

The EMSA was performed based on the method described previously (8). Synthesized oligonucleotides, 5′-GCTGTTTATTTCTGCTCTCCCTTG-3′ (corresponding to −330/−307 of the human FcεRIα gene with −315T) and 5′-GCTGTTTATTTCTGCCCTCCCTTG-3′ (−315C), labeled with FITC at the 5′-end were annealed with each complementary oligonucleotide labeled with FITC at the 5′-end. Nuclear extracts were prepared from PT18 and RBL-2H3 as previously described (9, 10). Abs against Sp1 (E3), Sp3 (F-7), MAZ (H-50), and YY1 (H-10) were purchased from Santa Cruz Biotechnology. For the competition assay, non-labeled dsDNA with the following sequences were used: Sp1 (5′-ATTCGATCGG GGCGGGGCGAGC-3′), MAZ (5′-CTCGCGCCCTCCCCCGC-3′), and control, a non-related sequence on the human FcεRI α-chain gene (5′-CTTTTGAGAATTCTACATGCTAC-3′).

Recombinant Sp1 protein was purchased from ProteinOne.com. Sp1 protein was also prepared using a TnT T7 Quick coupled transcription/translation system (Promega) using pCR-Sp1 (11), which was generated by insertion of human Sp1 cDNA into pCR3.1 (Invitrogen), as the template. All EMSA experiments were repeated more than three times; a typical result for each experiment is shown.

Purification of human basophils

Human basophils were purified from peripheral blood as previously described (8). In brief, human PBMC from 60 ml of peripheral blood was isolated by density gradient centrifugation and then treated with isotonic ammonium chloride buffer to lyse erythrocytes, followed by washing with running buffer (PBS supplemented with 0.5% BSA and 2 mM EDTA). To enrich the basophils present in the PBMC, the PBMC suspension was treated with reagents from Basophil Isolation kit II (Miltenyi Biotec), and separated using the autoMACS Separator (Miltenyi Biotec). The purity of the isolated basophils was confirmed to be ~95% by flow cytometry using anti-human FcεRI α-chain Ab, which does not compete with IgE (Cosmo Bio). The magnetically labeled cells, which were collected in the positive fraction, were used as non-basophils.

ChIP assay

The ChIP assay was performed using a ChIP Assay kit (Upstate Biotechnology) as in the previous report (7). Anti-Sp1 mouse IgG1 Ab (E6; Santa Cruz Biotechnologies), anti-HMG1 mouse IgG2b (clone 115603; R&D Systems), anti-HMG2 rabbit IgG Ab (BD Pharmingen), anti-HMG-I/HMG-Y goat IgG (N-19; Santa Cruz Biotechnologies), mouse IgG1 (BD Pharmingen), mouse IgG2b (BD Pharmingen), rabbit IgG (Sigma-Aldrich), and goat IgG (Sigma-Aldrich) were used. Quantitative PCRs were performed using TaqMan Universal PCR master mix (Applied Biosystems) and a 7500 Real-Time PCR system (Applied Biosystems). The following primers and TaqMan probe sequences were used for this analysis: for the promoter region of the α-chain gene including −315 (−366/−239), forward primer −366F (5′-ACCTGGCATATGTTTGTA

TTCAGT-3′), reverse primer −239R (5′-TCACAAGCCTTTCTTAATC TGTCAG-3′), and TaqMan probe −309T (5′-FAM-TTGCATCCCACTT TT-MGB-3′). The amount of target DNA bound to Sp1, HMG1, 2, or I/Y was quantified from the cycle threshold value, which was determined using 7500 SDS software (Applied Biosystems). In brief, the ratio of the amount of a specific DNA fragment in each immunoprecipitate to the amount of that fragment in the DNA before immunoprecipitation (input DNA) was calculated from each cycle threshold value.

Statistical analysis of −315 and −66 SNPs in the Japanese population

Genomic DNA was prepared from peripheral blood obtained from Japanese volunteers using a DNA quick kit (Dainippon Pharmaceutical) and was used as the template for PCR with the following primer set to amplify the promoter region of the FcεRIα gene (−551/+4); forward primer −551/−518 (5′-CTTAGGGGTTAGATTTTATGTGTTTGAACCCCAA-3′) and reverse primer +33/+4 (5′-CCATCTTCTTCATGGACTCCTG GTGCTTAC-3′). The nucleotide sequences of the DNA fragments, which were purified from the PCR product using Microcon YM-50 (Millipore), were determined with the ABI PRISM3100 Genetic Analyzer (Applied Biosystems) using a BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems) and a sequencing primer −441/−413 (5′-GTAGATAGTATGATGTACTTTATAGG-3′).

The D' and r^2 between −315 and −66 in FcεRIα were calculated using Haploview (version 3.2; downloaded from the HapMap site http://www.hapmap.org/cgi-perl/gbrowse/hapmap_B35/).

Measurement of FcεRI on peripheral blood basophils by flow cytometry

Basophils were purified from 5 to 15 ml peripheral blood by the MACS separation technique as described above. Purified basophils were analyzed by flow cytometry on a FACSCalibur (BD Biosciences) after staining with FITC-conjugated anti-human FcεRI α-chain Ab CRA1, which does not compete with IgE (Cosmo Bio), as described in our previous studies (2, 8). The p values were determined using a Mann-Whitney non-parametric test to assess differences in mean fluorescence intensity (MFI) of FcεRI α-chain between each genotype and a p value <0.05 was considered statistically significance. The calculation was performed with the SPSS software (version 11.0; SPSS).

Results

The −315T>C and −66T>C SNPs affect the transcription activity of human FcεRI α-chain promoter

To evaluate the effects of SNPs at −315 and −66 in the human FcεRI α-chain gene on transcription activity, we prepared four reporter plasmids carrying the luciferase gene under control of the human FcεRI α-chain promoter −605/+29 region. These four plasmids possess sequence variations at −315 and −66 of the α-chain promoter as shown in Fig. 1. Each of these reporter plasmids was transfected into the rat basophil cell line RBL-2H3, the mouse mast cell line PT18, and the human basophil cell line KU812, as the human FcεRI α-chain promoter has been shown to be activated in rodent cells in a manner similar to that in human cells (6, 10, 12, 13). The order of the luciferase activity strength driven by each promoter was roughly similar among RBL-2H3 (Fig. 1A), PT18 (Fig. 1B), and KU812 (Fig. 1C). In brief, the −315T/−66T promoter exhibited the highest promoter activity, whereas the −315C/−66C promoter showed the lowest activity. The other two promoters, −315C/−66T and −315T/−66C, resulted in intermediate strength activity. Thus, the −315T and −66T SNPs result in greater promoter activities than the −315C and −66C SNPs, respectively. The effect of the −66T>C SNP on promoter activity coincides well with our previous observation (2), although the role of −315C/T SNP was not discussed in that study. The comparable activity of the −315C/−66T and −315T/−66C promoters suggests that each of the SNPs at −315 and −66 contributes equally to promoter activity in vitro.

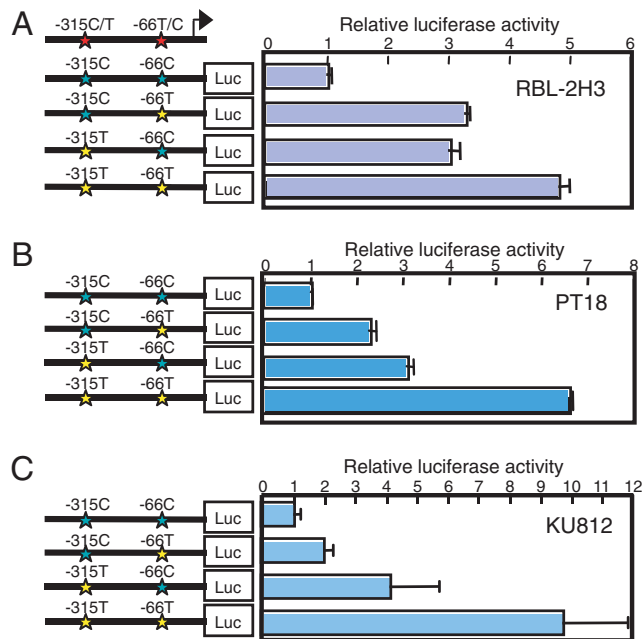


FIGURE 1. Effect of $-315C>T$ and $-66T>C$ SNPs on the transcription activity of human *FceRI* α -chain promoter. RBL-2H3 (A), PT18 (B), or KU812 (C) were transiently transfected with pGV-B2- α NN0.6- $-315C/-66C$, $-315C/-66T$, $-315T/-66C$, $-315T/-66T$, or pGL3-Basic. The relative luciferase activity is represented as the ratio of the activity to that of pGL3-Basic. Data represent the average $\pm$ SD of triplicate samples. A representative result of three independent experiments is shown.

Specific binding of nuclear proteins to the $-315C>T$ SNP site

GATA-1-mediated differential transactivation via $-66T>C$ has been indicated in previous studies (2). Similarly, the significant effect of the SNP at -315 on promoter activity suggests that different transcription factors might recognize each SNP. We then performed EMSA with nuclear extracts from RBL-2H3 and PT18 using double-stranded oligonucleotides ($-330/-307$) with $-315C>T$ SNP as the probe. When the mixtures of nuclear extracts from RBL-2H3 and probes with $-315T$ and $-315C$ were loaded on a polyacrylamide gel in side by side, the EMSA profile differed between the two probes, as observed in Fig. 2A. A similar profile with several different bands was also observed using the extracts from PT18 (data not shown). To evaluate the specific effect of this SNP on the band shifts in EMSA, competition assays with excess amounts of unlabeled double-stranded oligonucleotides were performed. When the $-315T$ allele probe was used, the band marked with an asterisk specifically disappeared by addition of excess unlabeled competitor with $-315T$ but not $-315C$ competitor or non-related sequence (Fig. 2B). This result suggests the presence of a nuclear protein that specifically bound to the $-315T$ allele. In similar experiments using the $-315C$ allele probe, a specific band shift marked with double asterisks (Fig. 2C) was identified. These results indicate that two different nuclear proteins discriminate the -315 SNP and are bound to either $-315T$ or $-315C$.

Identification of a nuclear protein binding to the $-315C$ sequence

We noticed that the nuclear substitution of C for T at -315 generated GC-rich sequence, which is a potential Sp1-binding motif, at this position. To examine the binding of Sp1 to the $-315C$ allele, we added anti-Sp1 Ab to the mixture of $-315C$ probe and nuclear proteins from RBL-2H3. As shown in Fig. 3A, the target

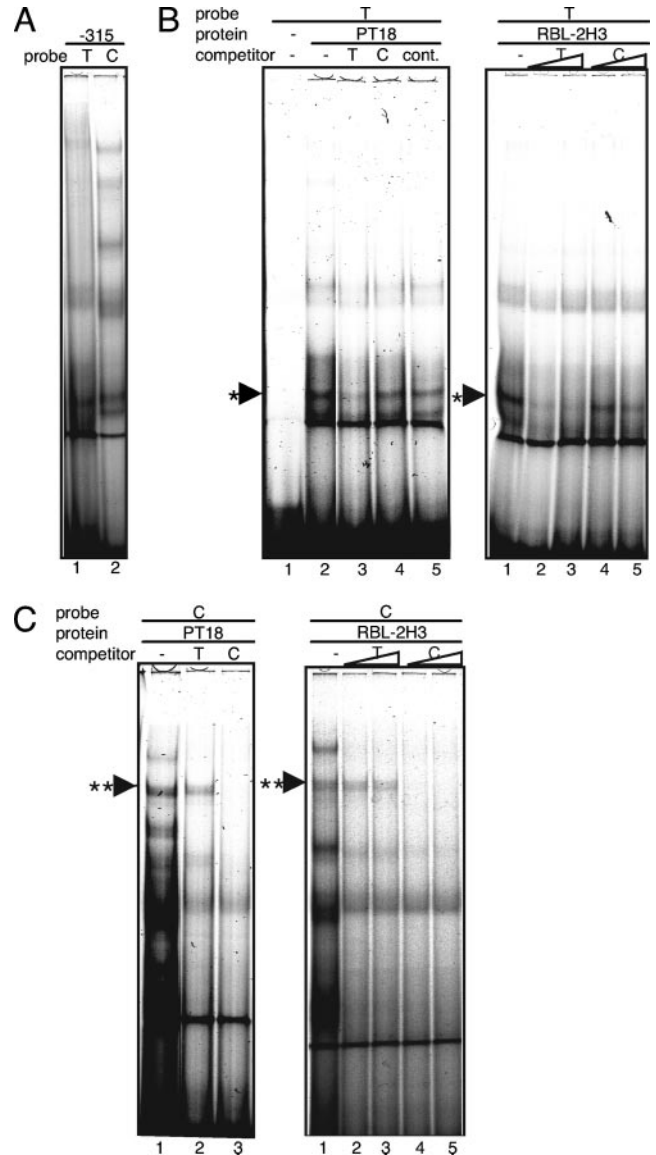


FIGURE 2. Transcription factors binding to the -315 SNP. A, Effect of $-315C>T$ SNP on the EMSA profile. EMSA was performed with FITC-labeled $-330/-307$ probes carrying $-315T$ (lane 1) and $-315C$ (lane 2) and nuclear extracts from RBL-2H3. All EMSA data in Figs. 2 and 3 are typical results obtained in one of three independent experiments. B, $-315T$ -specific binding. EMSAs were performed with a probe of $-315T$ and nuclear extracts from PT18 (left) and RBL-2H3 (right). For the competition assay in the left panel, 100-fold excess of non-labeled double-stranded oligonucleotides carrying T at -315 (lane 3), C at -315 (lane 4), and non-related sequence of the α -chain gene (lane 5) were added. In the right panel, 100- and 200-fold of $-315T$ (lanes 2 and 3) and $-315C$ (lanes 4 and 5) competitors were used. The specific band noted in the text is indicated with an asterisk. C, $-315C$ -specific binding. EMSAs were performed with a probe of $-315C$ and nuclear extracts from PT18 (left) and RBL-2H3 (right). The amount of competitive oligonucleotides was the same as for the $-315T$ probe (B). The specific band noted in the text is indicated with double asterisks.

band marked with double asterisks was supershifted by addition of anti-Sp1 Ab but was not affected by addition of Ab against another Sp1-family protein, Sp3, and irrelevant anti-YY1 Ab. In addition, Ab against MAZ, which was recently reported by Bae et al. (5), to bind the $-315C$ allele probe exhibited no effect on this specific band (Fig. 3A). This result indicates that the specific band with the $-315C$ oligonucleotide is a complex with Sp1. Furthermore, when

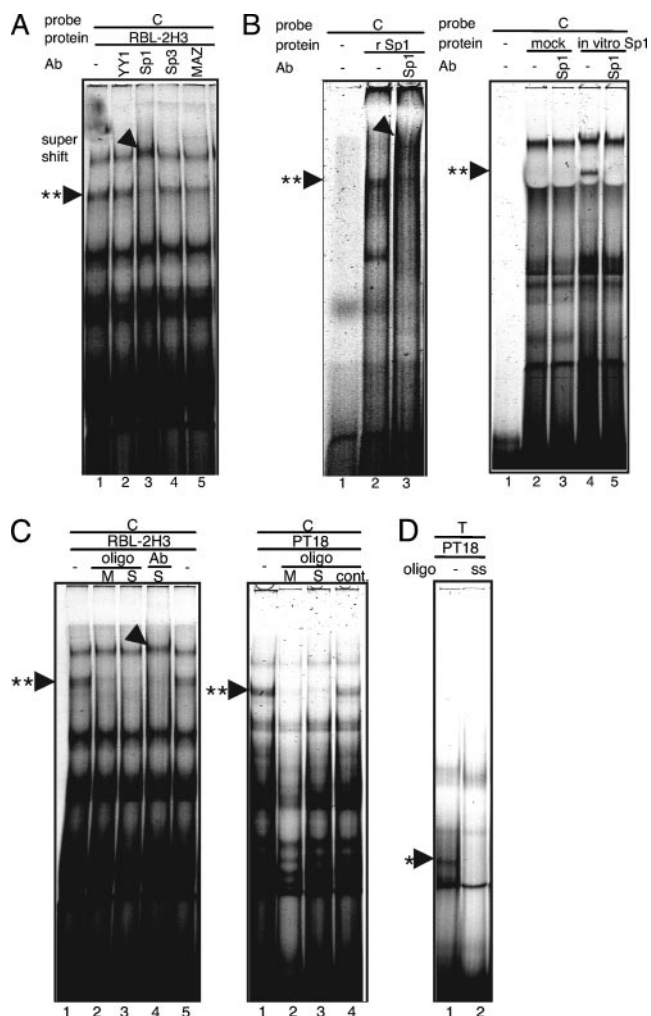


FIGURE 3. Identification of nuclear proteins binding to $-315C$ and $-315T$ by EMSA. **A**, Identification of a protein forming a $-315C$ -specific complex. The mixture of the $-315C$ probe and nuclear extract from RBL-2H3 was applied into each well after addition of each Ab. Lane 1, without Ab; lane 2, with anti-YY1 Ab; lane 3, with anti-Sp1 Ab; lane 4, with anti-Sp3 Ab; lane 5, with anti-MAZ Ab. The specific band noted in the text is indicated with double asterisks and the supershift band is marked with an arrowhead (in A, B, and C). **B**, Binding of rSp1 (left) and in vitro translated Sp1 (right) to the $-315C$ probe (left). The $-315C$ probe was mixed with human rSp1 (lanes 2 and 3 in left) or in vitro translated Sp1 (lanes 4 and 5 in right) in the presence (lane 3 in left and lane 5 in right) or absence (lane 2 in left and lane 4 in right) of anti-Sp1 Ab. **C**, Effect of transcription factor-specific competitive oligonucleotides on the $-315C$ -specific band. EMSAs were performed with the $-315C$ probe and nuclear extracts from RBL-2H3 (left) and PT18 (right). Competitive oligonucleotides containing MAZ-binding sequence (lane 2 in both panels), Sp1-binding sequence (lane 3 in both panels), and non-related sequence (lane 4 in right) were added to the probe-protein mixtures. **D**, Effect of competitive oligonucleotides on the $-315T$ -specific complex. The mixture of the $-315T$ probe and nuclear extract from PT18 were loaded in the presence (lane 2) or absence (lane 1) of single-stranded oligonucleotides of sense sequence of $-315T$.

recombinant Sp1 or in vitro-translated Sp1 was used instead of nuclear extracts, the specific band marked with double asterisks appeared and the band disappeared with the addition of anti-Sp1 Ab (the supershift band of the in vitro-translated Sp1 was not detected probably due to overlapping with a non-specific band) (Fig. 3B), indicating that the $-315C$ probe possesses the Sp1-binding sequence. In their study, Bae et al. (5) performed a competition

assay with oligonucleotides carrying specific binding sequences for transcription factors to identify the $-315C$ -specific band. To confirm the effect of reported competitors, we performed a competition assay using the same competitors for MAZ and Sp1 as that previous report. When oligonucleotides with the MAZ-binding sequence were added to the EMSA mixture, the specific band marked with double asterisks disappeared as is in their previous report (lane 2 in both panels of Fig. 3C). However, oligonucleotides with the Sp1-binding sequence, which did not affect the band profile in their report, also caused the disappearance of the specific band in the present study (lane 3 in both panels of Fig. 3C). Thus, we believe that specific binding of Sp1 to the $-315C$ SNP is hampered by MAZ oligonucleotides that also contain the Sp1-recognizable sequence.

Identification of a nuclear protein binding to the $-315T$ sequence

The specific band of the $-315T$ probe has quite high mobility, suggesting the possibility that this band is a complex of the probe and a nuclear protein belonging to an HMG-family. HMG proteins bind not only dsDNA but also ssDNA and induce conformational changes in the DNA. To examine this possibility, we added single-stranded oligonucleotides into the EMSA mixture instead of a double-stranded competitor. Addition of a single-stranded oligonucleotide with the sense sequence of the $-315T$ probe caused disappearance of the specific band (lane 2 in Fig. 3D). This observation suggests that an HMG-family protein that can specifically recognize the sense strand binds the $-315T$ allele DNA.

Binding of transcription factors on -315 SNP site in vivo

To examine whether any HMG-family proteins bind to the promoter region of the *FcεRIα* gene with the -315 T allele, a quantitative ChIP assay was performed, because EMSA with various Abs against HMG-family proteins resulted in somewhat of a reduction through not complete disappearance of the corresponding band. The human basophil cell line KU812, which was confirmed to be C/T heterozygous at -315 by sequencing analysis, was used for this ChIP assay. When immunoprecipitation was performed with Abs against HMG1 and 2, a higher amount of chromatin containing the α -chain promoter ($-311/-239$) was detected in the precipitated DNA compared with control Abs (Fig. 4A). In contrast, the amount of α -chain promoter genomic DNA precipitated with anti-HMG-I/Y Ab was lower than that with control Ab (Fig. 4A). Given these observations, we concluded that HMG1 and 2 bind to α -chain promoter with a higher affinity than that of HMG-I/Y.

To confirm the binding of Sp1 to $-315C$ in vivo, a quantitative ChIP assay using human basophils prepared from the peripheral blood obtained from an individual with the C/C genotype was done (Fig. 4B, left). In the cells of the basophil-enriched fraction ($\sim 95\%$ was FcεRIα-positive on FACS analysis), a significantly higher amount of the α -chain promoter region was immunoprecipitated by anti-Sp1 Ab compared with control mouse IgG1. When basophils from three individuals of $-315C/C$ -genotype were used, similar result was obtained (Fig. 4B, right). This suggests that Sp1 binds to the promoter region in living human basophils (Fig. 4B). In addition, the binding of Sp1 to the α -chain promoter was not observed in the non-basophil fraction (Fig. 4B, left). These results indicate that Sp1 binds to the $-315C/C$ -genotyped α -chain promoter in a basophil-specific manner.

Frequencies of -315 and -66 SNPs in the Japanese population

The above-mentioned reporter assay result suggests that both SNPs at -315 and -66 equally affect α -chain promoter activity

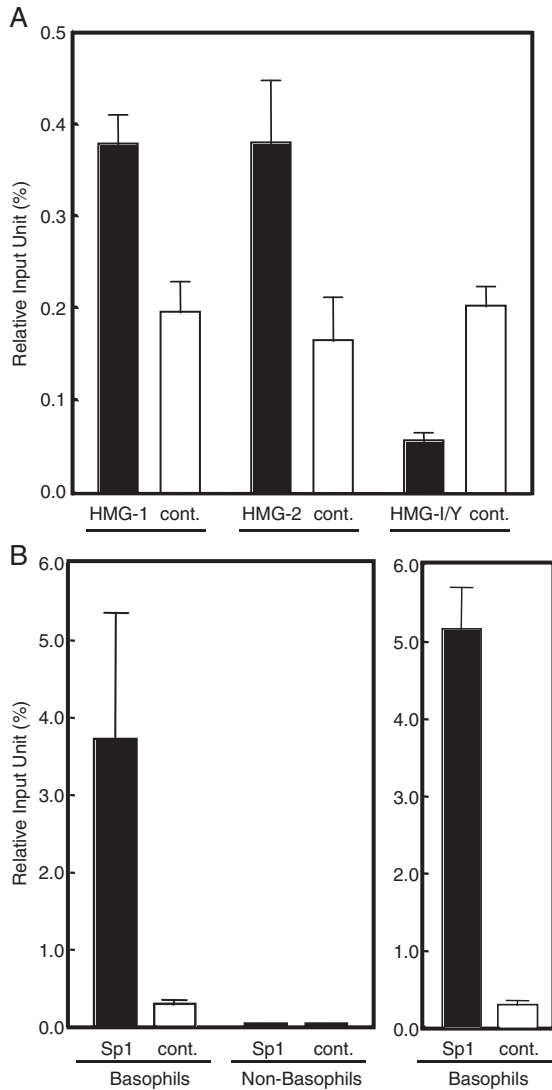


FIGURE 4. In vivo binding of HMG-family proteins and Sp1 to α -chain promoter in Fc ϵ RI α -chain expressing cells. Quantitative analysis of HMG-family proteins (A) or Sp1 (B) binding to the promoter region (–366/–239) of the α -chain gene using the ChIP assay. The results are expressed as mean + SD for two or three PCR with duplicate samples for each of three independent ChIPs using KU812 (A), for two PCR with duplicate samples for each of two independent ChIPs using basophil and non-basophil fractions prepared from 60 ml blood of the same individual carrying C/C genotype at –315 on different days (left in B), or three PCR with duplicate samples using basophils from 30 ml each blood of three individuals carrying –315C/C (right in B). Closed bar, specific Ab; open bar, control Ab.

(Fig. 1). Previously, several statistical analyses showed a positive association between –315 SNP (3–5) or –66 SNP (2) and allergic diseases. These observations indicate that both SNPs are involved

Table I. Genotypes of –315 and –66 SNPs on Fc ϵ RI α in the Japanese population

	–66			Total
	T/T	T/C	C/C	
–315				
C/C	19	4	1	24
C/T	15	1	0	16
T/T	6	0	0	6
Total	40	5	1	46

Table II. Frequencies of haplotypes in the Japanese population^a

Haplotypes		Number of Alleles	Ratio
–315	–66		
C	T	57	0.63
T	T	27	0.30
C	C	6	0.07
T	C	0	0.00
Total allele		90	

^a Allelic frequencies were calculated from data of Table I after removing one individual carrying heterogous genotypes on both SNPs.

in allergic diseases by affecting promoter activity. However, the relationship between –315 and –66 SNP is controversial, which may reflect statistical interdependence. In brief, –315 and –66 SNPs were described to be in complete linkage disequilibrium (LD) in one report (4) but not in LD in another one (5). Therefore, in the present study, we determined the genotypes of –315 and –66 in the Japanese population to examine the relationship of the two SNPs. In the Japanese population, a genotype C/C-T/T carrying major homo at both SNP sites was found with the highest frequency (41%), whereas three genotypes, C/T-C/C, T/T-T/C, and T/T-C/C, were absent (Table I). The parameters of LD calculated from Table I were $D' = 1.0$ and $r^2 = 0.036$. The order of haplotype frequencies was as follows: –315C/–66T > –315T/–66T > –315C/–66C, and –315T/–66C was absent (Table II). These results suggest that the LD between –315 and –66 is not in complete concordance, as shown by the r^2 value, due to the presence of three haplotypes, but that the two loci have a relationship without recombination as indicated by the full value of D' and by the absence of a –315T/–66C haplotype.

Positive association between –315 SNP and cell surface expression level of Fc ϵ RI

To analyze the effect of –315 SNP on cell surface expression level of Fc ϵ RI, we compared the amount of Fc ϵ RI on basophils among –315T/T ($n = 3$), –315T/C ($n = 7$), and –315C/C ($n = 13$)

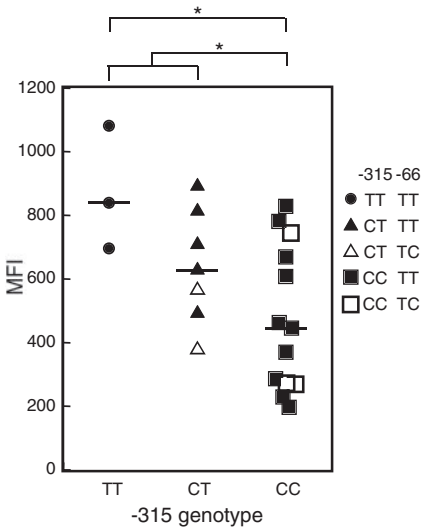


FIGURE 5. Cell surface expression level of Fc ϵ RI on freshly purified basophils from peripheral bloods of volunteer individuals. Genotypes of –315 and –66 SNPs are as follows: closed circle, T/T-T/T; closed triangle, C/T-T/T; open triangle, C/T-T/C; closed square, C/C-T/T; open square, C/C-T/C. Bars mean medians in each group of –315 genotype. *, $p < 0.05$, as determined by a Mann-Whitney non-parametric test.

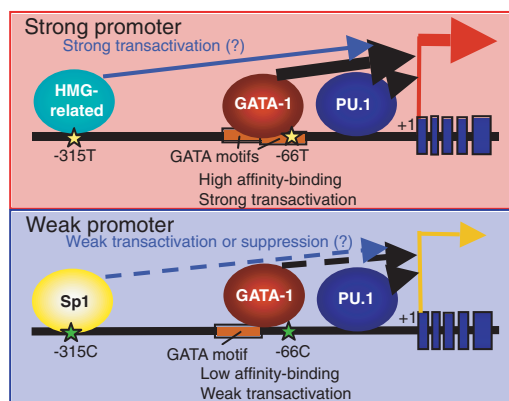


FIGURE 6. Schematic drawing of the human *FcεRI* α -chain promoter structure. Typical strong promoter carrying $-315T$ and $-66T$ (top panel) and typical weak promoter carrying $-315C$ and $-66C$ (bottom panel) are shown. The α -chain promoter with T at -66 has an additional GATA-1-binding motif in the region, which assures higher affinity of GATA-1 to the promoter, resulting in higher transcriptional activity of the promoter (2). In the present study, different binding of transcription factors to the -315 -SNP site was found. Sp1 and HMG-family proteins (HMG1 and/or 2) bind to the $-315C$ and $-315T$ sequences, respectively.

individuals (Fig. 5). Significant differences were observed between $-315T/T$ vs $-315C/C$ ($p = 0.025$) and between ($-315T/T$ plus $-315T/C$) vs $-315C/C$ ($p = 0.021$), suggesting that basophils carrying $-315T$ allele express higher amount of *FcεRI* on surface rather than $-315C/C$ basophils.

Discussion

The *FcεRI* α -chain is essential for functional expression of *FcεRI* on the cell surface and binding to IgE Ab. The necessity of the α -chain in IgE-mediated allergic reactions was definitively proved by the abolishment of anaphylaxis in α -chain-deficient mice (1). Recently, several groups including us reported studies regarding the association of -315 and -66 SNPs in the α -chain promoter with allergic diseases (2–5). In the present study, we analyzed the role of these SNPs in the function of the α -chain promoter and found that the $-315C$ and $-315T$ sequences are recognized by two different transcription factors, as summarized in Fig. 6. During the preparation of this manuscript, Bae et al. (5) reported that the transcription factor MAZ binds to $-315C$. Although we also found specific binding of a nuclear protein to the $-315C$ probe in the present study, it was identified as Sp1 and not MAZ, based on the following findings: i) the specific band was supershifted by anti-Sp1 Ab, ii) recombinant Sp1 and in vitro-translated Sp1 caused the same band shift of the probe in EMSA (Fig. 3, A–C), and iii) the ChIP assay showed that Sp1 bound around $-315C$ of genomic DNA in basophils in vivo (Fig. 4B). MAZ is a transcription factor that recognizes the sequence CCCACCC. A similar sequence overlaps with the putative Sp1-recognition sequence in the α -chain promoter $-315C$ sequence, which may explain why the transcription factor was mistakenly identified as MAZ by Bae et al. (5).

The α -chain promoter carrying $-315T$ possessed higher transcription activity than that carrying $-315C$, irrespective of the SNP at position -66 (Fig. 1). Bae et al. (5) discussed the possibility that MAZ acts as a repressor on the α -chain promoter carrying $-315C$, which exhibits lower transcription activity. In the present study, we demonstrated that $-315C$ is recognized by Sp1 but not MAZ. Therefore, the repression hypothesis must be reconsidered, because Sp1 is well known as a transactivator, not a repressor. The higher activity of the promoter with binding of HMG-

family proteins around the -315 site transactivates the promoter much higher than that of Sp1. In any case, it turned out to be true that the -315 SNP critically affects promoter activity, and preferential binding of different transcription factors, Sp1 to $-315C$ and HMG-family proteins to $-315T$, is involved in the expression level of α -chain.

HMG-family proteins are architectural transcription factors that function as components of chromatin structure and as auxiliary of other transcription factors. In brief, HMG proteins cause DNA bending and facilitate the binding of several regulatory protein complexes to DNA. There are several studies that indicate the involvement of HMG proteins in the regulation of immunorelated genes including V(D)J recombination, IFN- β , IL-2, IL-2R α , IL-4, GM-CSF, and TNF- β (14–16). From these observations, we may think that an HMG-family protein transactivates the human *FcεRI* α -chain promoter. The binding of HMG1 and 2 but not HMG-I/Y to α -chain promoter was detected in the ChIP assay using KU812 carrying C/T heterozygous at -315 . This suggests that HMG1 and/or 2 bind around -315 of *FcεRI* α genomic DNA in vivo. Considering the quite high homology between HMG1 and 2 (84%), it may be difficult to distinguish these two HMG-family proteins, which probably recognize the same region via their highly conserved DNA-binding domain. Although further detailed analysis is required to clarify the involvement of HMG1 and/or 2 in *FcεRI* α -chain expression, these HMG-family proteins bind to the promoter region including the $-315T$ sequence in living cells.

We have previously indicated that a $-66T>C$ -SNP, which affects promoter activity via different binding affinity to GATA-1, associates with atopic dermatitis in allele frequency and with *FcεRI* expression levels, without considering a $-315C>T$ -SNP (6). In contrast, Shikanai et al. (3) showed that a -315 SNP (-335 in their report) is associated with IgE levels in Caucasian asthmatic patients, without considering a $-66T>C$ -SNP. In the present study, we found that promoter activities of $-315C/-66T$ and $-315T/-66C$ are intermediate between those of $-315T/-66T$ and $-315C/-66C$ (Fig. 1), suggesting that the effect of both SNPs on α -chain promoter activity is almost equal in a basic molecular analysis. Recently, two independent groups reported that an SNP at -315 (-344) but not -66 (-95) is associated with IgE levels in allergic patients with asthma or urticaria (4), or IgE levels in aspirin-intolerant chronic urticaria (5). Interestingly, these two SNPs at -315 and -66 were not judged to be in LD in one report (5), whereas they were concluded to be in complete LD in another one (4, 17). The latter study showed allele frequency including both SNPs; in brief, $-315T/-66T$ is the most common, the two haplotype variants $-315C/-66T$ and $-315C/-66C$ occur with similar frequency, and $-315T/-66C$ is absent in Polish population (4, 17). If the two SNPs are linked to each other, the result of comparison between $-315T$ and $-315C$ may be partly influenced by -66 SNP. It was found that the haplotype $-315T/-66C$ was absent in the Japanese population (Table II) as well as in a Polish case (4). This haplotype has not been found in our subsequent study involving >200 additional individuals to date (data not shown). Therefore, the statistical analysis of -315 SNP may be partly affected by -66 SNP, because the absence of $-315T/-66C$ suggests the possibility that a comparison of $-315T$ vs $-315C$ is a comparison of ($-66T$ alone) vs ($-66T$ plus $-66C$). When expression level of *FcεRI* on basophils was analyzed, -315 SNP was significantly associated with MFI (Fig. 5). This is the first report showing association between -315 and *FcεRI* expression level. In addition, medians of MFI of $-66T/C$ were lower than those of $-66T/T$: in brief, 477 ($-315C/T$ and $-66T/C$; open triangle) vs 712 ($-315C/T$ and $-66C/C$; closed triangle), and 272 ($-315C/C$ and $-66T/C$; open square) vs 449 ($-315C/C$ and $-66T/T$; closed

square). This observation is the same tendency as in our previous study (2). To further clarify the contribution of both SNPs to allergic diseases, a statistical analysis considering the relationship between the two SNPs is in progress.

The gene of β -chain, another component of FcεRI, possesses SNPs affecting transcription activity and associated with cell surface expression level of FcεRI (8). Comparison of cell surface level of FcεRI considering SNPs on α - and β -chain genes may be useful to clarify the involvement of each SNP on FcεRI expression. This analysis will be performed using larger population with enough number of rare alleles.

In several reports, SNPs in the promoter region of genes encoding FcεRI α - and β -chains were shown to be associated with serum IgE levels of allergic patients (3–5, 8). We also found that serum IgE levels were associated with -315 SNP between C/C (median = 87 IU/ml) vs (C/T plus T/T) (median = 19 IU/ml) with same individuals as used in Fig. 5 ($p = 0.029$, data not shown). To date, the molecular mechanism for the elevated level of IgE in allergic patients carrying SNPs on regulatory regions of FcεRI genes is largely unknown, although the effect of the high amount of IgE on the high cell surface expression level of FcεRI is well characterized to be due to the increased stability of the FcεRI-IgE complex (18). Revealing the mechanism for controlling serum IgE level by SNPs on genes encoding FcεRI components may be important for providing useful information for prevention and/or treatment of allergic diseases.

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Disclosures

The authors have no financial conflict of interest.

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